

10/500, 301 AEB
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Atty. Dkt. No. 036910-0111

from the coding region of the CUC1 gene was designed to be in-frame with the reading frame of the coding region of SRD.

45 15 AEB 5/17/07
Please delete the paragraph on page 46, lines 24-27, and replace it with the following paragraph:

Accordingly, a peptide having the amino acid sequence DLDLELRGFA (SEQ ID NO: 119) (corresponding to the 194/204 repression domain of SUP) and a gene encoding the same peptide were found to be capable of converting any transcription factor into a transcriptional repressor.

45 19 AEB 5/17/07
Please delete the paragraph on page 46, line 28, to page 47, line 3, and replace it with the following paragraph:

(Example 6) Effects of gene encoding the peptide LDLELRGFA (SEQ ID NO: 16) (corresponding to the 195/204 repression domain of SUP (SRD1)) and the peptide LDLNLAPPEF (SEQ ID NO: 4) (corresponding to the 215/225 repression domain of ERF3 (RD1)) in repressing of the transcription activated by EIN3 in plants

46 23 AEB 5/17/07
Please delete the paragraph on page 48, lines 4-11, and replace it with the following paragraph:

Two DNA strands, each of which had been designed to encode the amino acid sequence of SRD1 (LDLELRGFA) (SEQ ID NO: 16) having the restriction site of *Sal*I at the 3' terminus and to have the amino acid reading frame to be in-frame with that of GAI 4DBD, were prepared in the same manner as in Example 2 (2) (2-2).

5'-CCTGGATCTAGAACTCCGTTTGGGTTTCGCTTAA-3' (SEQ ID NO: 57)

5'-TCGACTTAAGCGAAACCCAAACGGAGTTCTAGATCCAGG-3' (SEQ ID NO: 58)

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Please delete the paragraph on page 48, lines 14-21, and replace it with the following paragraph:

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~~on Aeg 5/17/07~~
Please delete the paragraph on page ~~28~~, lines ~~2-7~~, and replace it with the following paragraph:

According to the above results, activity of reporter gene of a peptide comprising LDL(N/E)L (SEQ ID NO: 128) or FDLN(F/I) (SEQ ID NO: 129) and at least 6 amino acid residues at its C-terminus or a peptide comprising DL(E/Q/D)LRL (SEQ ID NO: 130) decreases by 85% to 98%, in comparison with that of the reporter gene (containing no effector plasmid, i.e., the control). This demonstrates that the aforementioned peptides serve as functional peptides capable of repressing transcriptions of genes.

~~44 4 Aeg 5/17/07~~
Please delete the paragraph on page ~~42~~, lines ~~7-12~~, and replace it with the following paragraph:

A DNA fragment of the nucleotide sequence (the 571/675 region) corresponding to the region encoding the amino acid sequence (the 191/225 region) of ERF3 having the restriction enzyme, Sall site at its 3' terminus was designed to have a reading frame to be in-frame with the carboxyl terminus of EIN3. The nucleotide sequence of the full-length ERF3 gene and the amino acid sequence thereof are shown in SEQ ID NOS: 53 and 133, respectively.

~~43 24 Aeg 5/17/07~~
Please delete the paragraph on page ~~45~~, lines ~~2-4~~, and replace it with the following paragraph:

(Example 5) Effects of gene encoding the peptide DLDLELRLGFA (SEQ ID NO: 119) (corresponding to the 194/204 repression domain of SUP (SRD)) in repressing functions of CUC1 to activate transcription in plants

~~44 5 Aeg 5/17/07~~
Please delete the paragraph on page ~~45~~, lines ~~9-16~~, and replace it with the following paragraph:

The following complementary strands (3' complement DNA) were synthesized to prepare the amino acid sequence (VSVWPFTLDLDLELRLGFA) (SEQ ID NO: 121). In this amino acid sequence, the amino acid peptide DLDLELRLGFA (SEQ ID NO: 119) (referred to as "SRD") was bound to the carboxyl terminus of the protein-encoding region (SEQ ID NO: 54 and encoded protein SEQ ID NO: 134) of the cup-shaped cotyledon 1 (CUC1) transcription factor. Also, the reading frame of the sequence in which the stop codon had been deleted

the transgenic plants. The effects of repressing the transcription of an anthocyanin-synthesizing gene in the plants were inspected.

18 18 Aeg 5/12/07
Please delete the paragraph on page 18, lines 3-12, and replace it with the following paragraph:

In Example 8, a chimeric repressor (35S::AtMYB23SRDX) prepared by applying a peptide (SRDX) consisting of 12 amino acid residues represented by the amino acid sequence LDLDLELRGFA (SEQ ID NO: 120) to the carboxyl terminus of the AtMYB23 transcription factor (Kirik V., Schnittger A., Radchuk V., Adler K., Hulskamp M., & Baumlein H., Ectopic expression of the Arabidopsis AtMYB23 gene induces differentiation of trichome cells, Dev Biol. 235, 366 (2001); a conserved MYB regulator of phenylpropanoid biosynthesis, Plant Cell 12, 2383 (2000)) was introduced to Arabidopsis thaliana to produce the transgenic plants. The effects of repressing the transcription of genes that regulate trichome generation were inspected.

20-27 Aeg 5/12/07
Please delete Table 1 on pages 27-28, and replace it with the following table:

Fig. 4A shows the structure of the reporter gene and that of the effector plasmid. Fig. 4B and Table 1 below show the results of assaying of activity of reporter gene.

(Table 1)

Identification of peptide	Peptide sequence	Relative value (%)	SEQ ID NO: _____
ERF3RD(214/225)	DLDLNLAPPMEF	15.0	<u>1</u>
3RD1	LDLNLAPPMEF	14.6	<u>4</u>
3RD2	LDLNLAAAAAA	17.5	<u>7</u>
SRD1	LDLELRGFA	2.0	<u>16</u>
SRD2	LDLELGFA	221	<u>19</u>
LELDL	LELDLAAAAAA	196	<u>22</u>
Min-LDLN	LDLN	153	<u>10</u>
Min-LDLNL	LDLNL	150	<u>13</u>
	LELRLLLLAAAA	130.6	<u>80</u>
	DLELR	8.9	<u>83</u>
	LDLQLRLGYY	3.8	<u>86</u>
	LDLELR	4.5	<u>89</u>
	LDLELAAAAAA	72.5	<u>92</u>
	LDLELRLLLL	6.9	<u>95</u>
	LELDLAAAAAA	196.0	<u>98</u>
	LDLELRG	8.9	<u>101</u>
	LELR	101.5	<u>104</u>
	FDLNFAPLDCV	17.5	<u>107</u>
	FDLNIFPPIPEF	16.0	<u>110</u>
	FQFDLNFPPPLDCV	10.9	<u>113</u>
	DLDLRL	9.2	<u>116</u>
Control	GAL4DB alone	100	

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Please delete the paragraph on page 16, lines 16-20, and replace it with the following paragraph:

This mechanism of repressing transcription is described in greater detail with reference to a case where the *Arabidopsis thaliana* ethylene-insensitive 3 gene (hereafter referred to as the "EIN3 gene") is used as a transcription factor. The sequence of this EIN3 gene and that of a protein produced there from are shown in SEQ ID NOS: 52 and 132, respectively.

17 25 AEG 5/17/07
Please delete the paragraph on page 16, lines 9-16, and replace it with the following paragraph:

In Example 5, a gene fragment encoding DLDLELRGFA (SEQ ID NO: 119) (SUPERMAN repression domain (SRD), residues 194-204) was fused to a transcription factor CUC1, and the resultant was ligated to the downstream region of the cauliflower mosaic virus 35S promoter to construct a plasmid for transformation, *Arabidopsis thaliana* was transformed using the aforementioned plasmid, and morphological changes of the cotyledon after germination were observed. Thus, effects of the aforementioned gene fragment in repressing the expression of the genes for CUC1, and for CUC2 that is functionally redundant with CUC1, were investigated

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Please delete the paragraph on page 18, lines 17-22, and replace it with the following paragraph:

In Example 6, a gene encoding LDLELRGFA (SEQ ID NO: 16) (SUPERMAN repression domain (SRD1), residues 195-204) and LDNLAPPMEF (SEQ ID NO: 4) (ERF3 repression domain (RD1), residues 215-225) was fused to a plant transcription factor EIN3, *Arabidopsis thaliana* was transformed in the same manner, and morphological changes of relevant plants in the presence of ethylene were observed. Thus, effects of the aforementioned gene fragment in repressing transcription of the target gene for EIN3 were inspected.

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Please delete the paragraph on page 18, line 28, to page 19, line 2, and replace it with the following paragraph:

In Example 7, a chimeric repressor (35S::PAP1SRDX) prepared by applying a peptide (SRDX) consisting of 12 amino acid residues represented by the amino acid sequence LDLELRGFA (SEQ ID NO: 120) to the carboxyl terminus of the production-of-anthocyanin-pigment 1 transcription factor (PAP1) (Borevitz J. O., Xia Y., Blount J., Dixon R. A. & Lamb C., Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis, *Plant Cell* 12, 2383, 2000) was introduced into *Arabidopsis thaliana* to produce

13 14 14 Aeg 5/17/07
Please delete the paragraph on page 13, line 23, to page 14, line 24, and replace it with the following paragraph:

This is described more specifically with reference to a case where the cup-shaped cotyledon 1 (CUC1) transcription factor is used (Plant Cell, 9, 841, 1997).

CUC1 is a transcription factor that regulates apical bud formation of seedlings together with CUC2 having the same NAC domain. Only when mutation is present in both CUC1 and CUC2 genes, the cotyledon of the plant forms a cup-like shape (a cup-shaped cotyledon), and the apical meristem is not formed. In contrast, a plant having mutation in only either CUC1 or CUC2 is normal. Accordingly, CUC1 and CUC2 are known to be functionally redundant factors (Development, 126, 1563, 1999; Development, 128, 1127, 2000). When a chimeric gene, in which a gene encoding the peptide of the present invention is bound to either one of the functionally redundant CUC1 or CUC2 transcription factor genes, for example, the CUC1 gene, is allowed to express in a plant, the expressed chimeric protein can suppress transcription activity of the functionally redundant CUC2 transcription factor as well as that of the CUC1 transcription factor. That is, it can repress the expression of genes regulated by the CUC1 transcription factor. In such a case, the cotyledon of the plant forms a cup-like shape, which is a trait of a *cuc1/cuc2* double mutant (a cup-shaped cotyledon), and the apical meristem is not formed. In the Example 5 below, a chimeric gene was constructed, wherein the gene encoding the DLDLELRGFA peptide (SEQ ID NO: 119) of the present invention (this peptide is referred to as "SRD") had been allowed to fuse with the CUC1 gene (Fig. 14), and *Arabidopsis thaliana* was transformed with the chimeric gene. This demonstrates that the transgenic plant takes on a cup-like shape indicating a phenotype of a *cuc1/cuc2* double mutant (a cup-shaped cotyledon) (Fig. 15, right). Formation of the apical meristem is not observed as with the case of the deficiencies of STM gene which regulates the formation of the apical meristem regulated by the CUC1 transcription factor. This indicates that the CUC1 transcription factor capable of activating transcription was functionally converted to a transcriptional repressor via fusion with the DLDLELRGFA peptide (SEQ ID NO: 119) of the present invention. The aforementioned further indicates that the above peptide suppresses not only activity of CUC1 transcription factor, but also preferentially suppresses activity of the CUC2 transcription factor which is functionally redundant with CUC1, and represses expression of genes located downstream.

Leu-Asp-Leu-Glu-Leu-Arg-Leu (SEQ ID NO: 89),

Leu-Asp-Leu-Glu-Leu-Arg-Leu-Gly (SEQ ID NO: 101),

Leu-Asp-Leu-Glu-Leu-Arg-Leu-Ala-Ala-Ala (SEQ ID NO: 95)

Leu-Asp-Leu-Glu-Leu-Arg-Leu-Gly-Phe-Ala (SEQ ID NO: 16)

Asp-Leu-Asp-Leu-Glu-Leu-Arg-Leu-Gly-Phe-Ala (SEQ ID NO: 119)

Leu-Asp-Leu-Asp-Leu-Glu-Leu-Arg-Leu-Gly-Phe-Ala (SEQ ID NO: 120)

14 Aeg 5/12/17
Please delete the paragraph on page 10, lines 20-22, and replace it with the following paragraph:

The peptide in the present invention that is capable of converting the transcription factor into a transcriptional repressor may comprise the minimum sequence Asp-Leu-Glu-Leu-Arg-Leu (SEQ ID NO: 83).

17 Aeg 5/12/17
Please delete the paragraph on page 10, lines 23-27, and replace it with the following paragraph:

In the above peptide, glutamic acid (E) in the minimum sequence may be substituted with glutamine (Q) or aspartic acid (D). Peptides, such as Leu-Asp-Leu-Gln-Leu-Arg-Leu-Gly-Tyr-Tyr (SEQ ID NO: 86) or Asp-Leu-Asp-Leu-Arg-Leu (SEQ ID NO: 116), have excellent effects of repressing transcriptional activities. In contrast, the sequence Leu-Glu-Leu-Arg-Leu (SEQ ID NO: 104) does not have a function of transcriptional repression.

11 21 Aeg 5/12/17
Please delete the paragraph on page 12, lines 11, and replace it with the following paragraph:

The SUP protein having the amino acid sequence as shown in SEQ ID NO: 31 and SUP gene are already known. The amino acid sequence (residues 195 to 199, corresponding to the nucleotide sequence (the 583/597 region)) are leucine (L)-aspartic acid (D)-leucine (L)-glutamic acid (E)-leucine (L) (SEQ ID NO: 127), and a proline residue is not present downstream of this sequence toward the 3'-terminus. Instead, an amino acid sequence that is different from the motif (L/F)DLN(L/F)(X)P (SEQ ID NO: 122) as mentioned in the Background Art section is present in the aforementioned sequence.

2 Aeg 5/17/07
Please delete the paragraph on page 9, lines 15-20, and replace it with the following paragraph:

The present invention also provides a peptide having the amino acid sequence represented by formula (II) and capable of converting a transcription factor into a transcriptional repressor:

Y1-Phe-Asp-Leu-Asn-Y2-Y3 (II) (SEQ ID NO: 124)

wherein Y1 denotes 0 to 10 amino acid residues; Y2 denotes Phe or Ile; and Y3 denotes at least 6 amino acid residues.

21 Aeg 5/17/07
Please delete the paragraph on page 9, line 27, to page 10, line 3, and replace it with the following paragraph:

The present invention also provides a peptide having the amino acid sequence represented by formula (III) and capable of converting a transcription factor into a transcriptional repressor:

Z1-Asp-Leu-Z2-Leu-Arg-Leu-Z3 (III) (SEQ ID NO: 125)

wherein Z1 denotes Leu, Asp-Leu, or Leu-Asp-Leu; Z2 denotes Glu, Gln, or Asp; and Z3 denotes 0 to 10 amino acid residues.

9 22 Aeg 5/17/07
Please delete the paragraph on page 10, lines 4-10, and replace it with the following paragraph:

In formula (III), Z3 may be 0 to 10 amino acid residues, although a shorter sequence is more convenient in terms of the ease of peptide synthesis. Thus, the number of amino acid residues is preferably 10 or less, and more preferably 5 or less. Specific examples of Z3 include, but are not limited to, G, GFF, GFA, GYY, and AAA. A peptide represented by formula (III) has a motif DLELRL (SEQ ID NO: 83) that is different from a conserved motif (L/F)DLN(L/F)(X)P (SEQ ID NO: 122) of peptides as described in the Background Art section. This motif corresponds to the amino acid sequence (the 196/201 region) of the SUP protein (Asp-Leu-Glu-Leu-Arg-Leu) (SEQ ID NO: 83). The total number of peptides is preferably 20 amino acids at a maximum in terms of the ease of peptide synthesis. Examples of preferable peptides include the following.

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Please delete the paragraph on page 7, lines 26-27, and replace it with the following paragraph:

Fig. 14 schematically shows the structure of the plasmid p35S::CUC1SRD for transforming *Arabidopsis thaliana* (SEQ ID NO: 119).

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Please delete the paragraph on page 8, lines 21-26, and replace it with the following paragraph:

The present invention provides a peptide having the amino acid sequence represented by formula (I) and capable of converting a transcription factor into a transcriptional repressor:

X1-Leu-Asp-Leu-X2-Leu-X3 (I) (SEQ ID NO: 123)

wherein X1 denotes 0 to 10 amino acid residues; X2 denotes Asn or Glu; and X3 denotes at least 6 amino acid residues.

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Please delete the paragraph on page 9, lines 4-9, and replace it with the following paragraph:

The number of amino acid residues denoted by X3 is essential, and a minimum of 6 amino acid residues was unexpectedly found to be sufficient for exhibiting the aforementioned functions. Further, X1 and X3 may be amino acids of any type. For example, P (proline) in the aforementioned conserved motif (L/F)DLN(L/F)(X)P (SEQ ID NO: 122) of peptides described in the Background Art section is not necessary for X3. X3 may be simply comprised of aligned alanines.

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Please delete the paragraph on page 9, lines 10-14, and replace it with the following paragraph:

In contrast, a sequence consisting of LDLNL (Leu-Asp-Leu-Asn-Leu) (SEQ ID NO: 13) or LDLN (Leu-Asp-Leu-Asn) (SEQ ID NO: 10) does not exhibit the aforementioned functions. A sequence that was designed to have 5 or 6 amino acid residues denoted by X2 very significantly exhibits the aforementioned functions whereas the one designed to have 3 amino acid residues does not exhibit such functions.

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Atty. Dkt. No. 081356-0219

Amendments to the Specification:

Please amend the title as follows:

GENE AND PEPTIDE FOR TRANSCRIPTIONAL REGULATORY GENE AND
PEPTIDE REPRESSOR

On page 1, directly beneath the title, ~~please insert the following~~ ^{Brief Description of Drawings} 5/17 Aeg

--The application contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.--